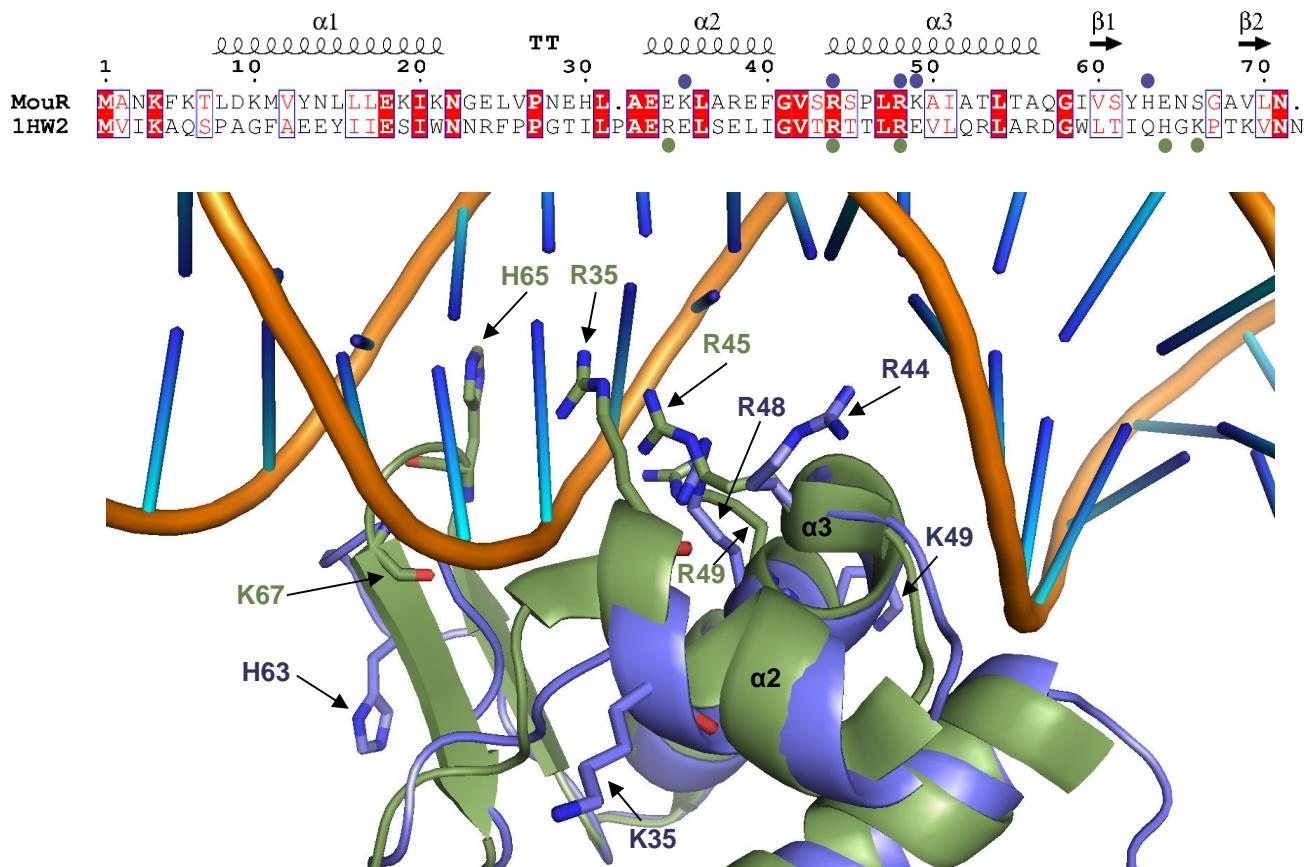
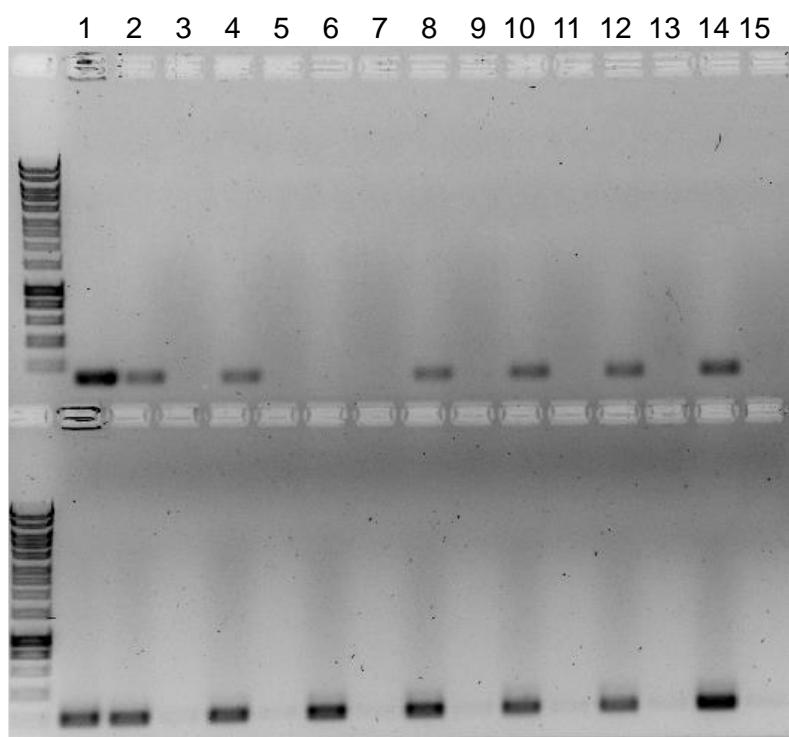


Supplementary Data

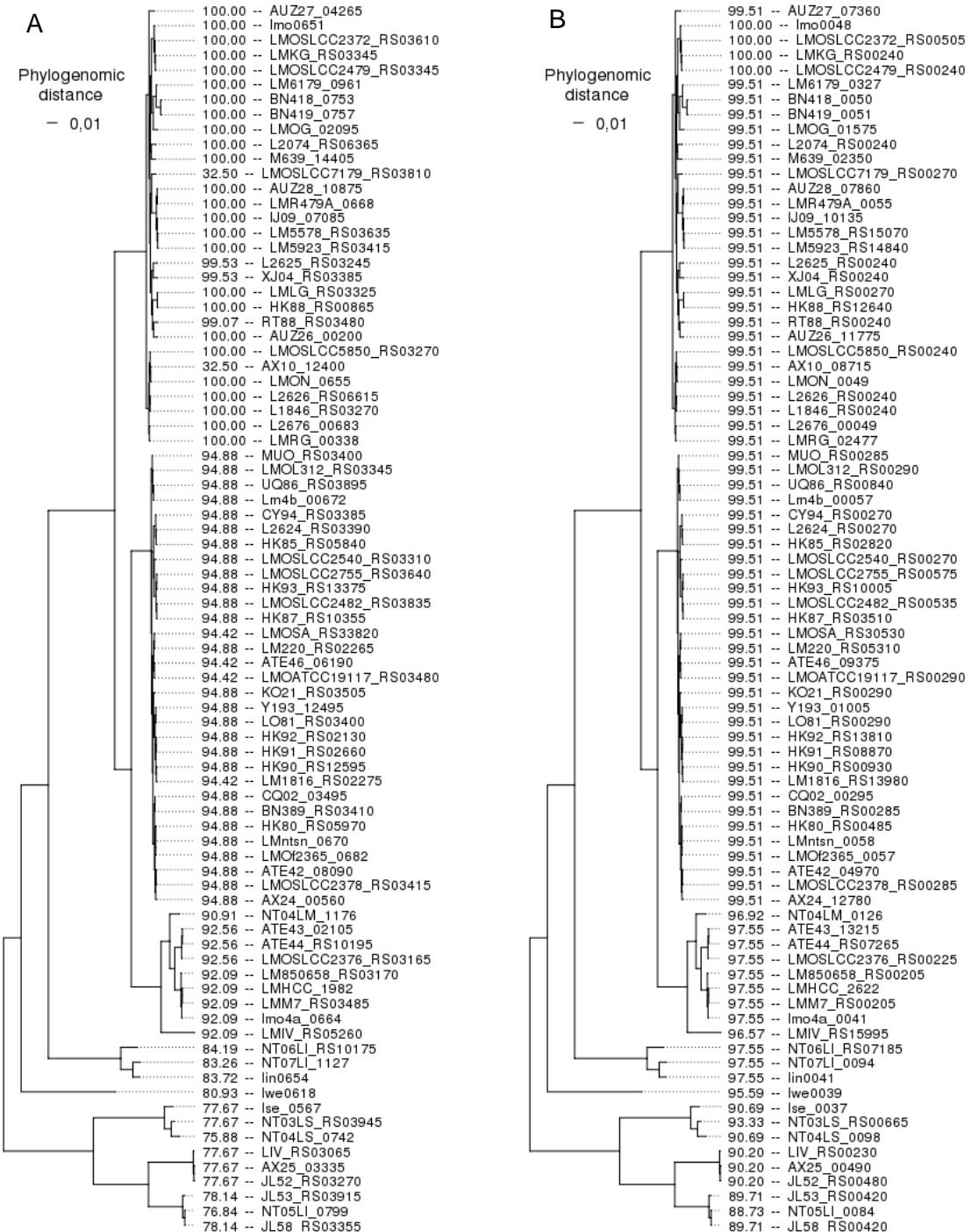


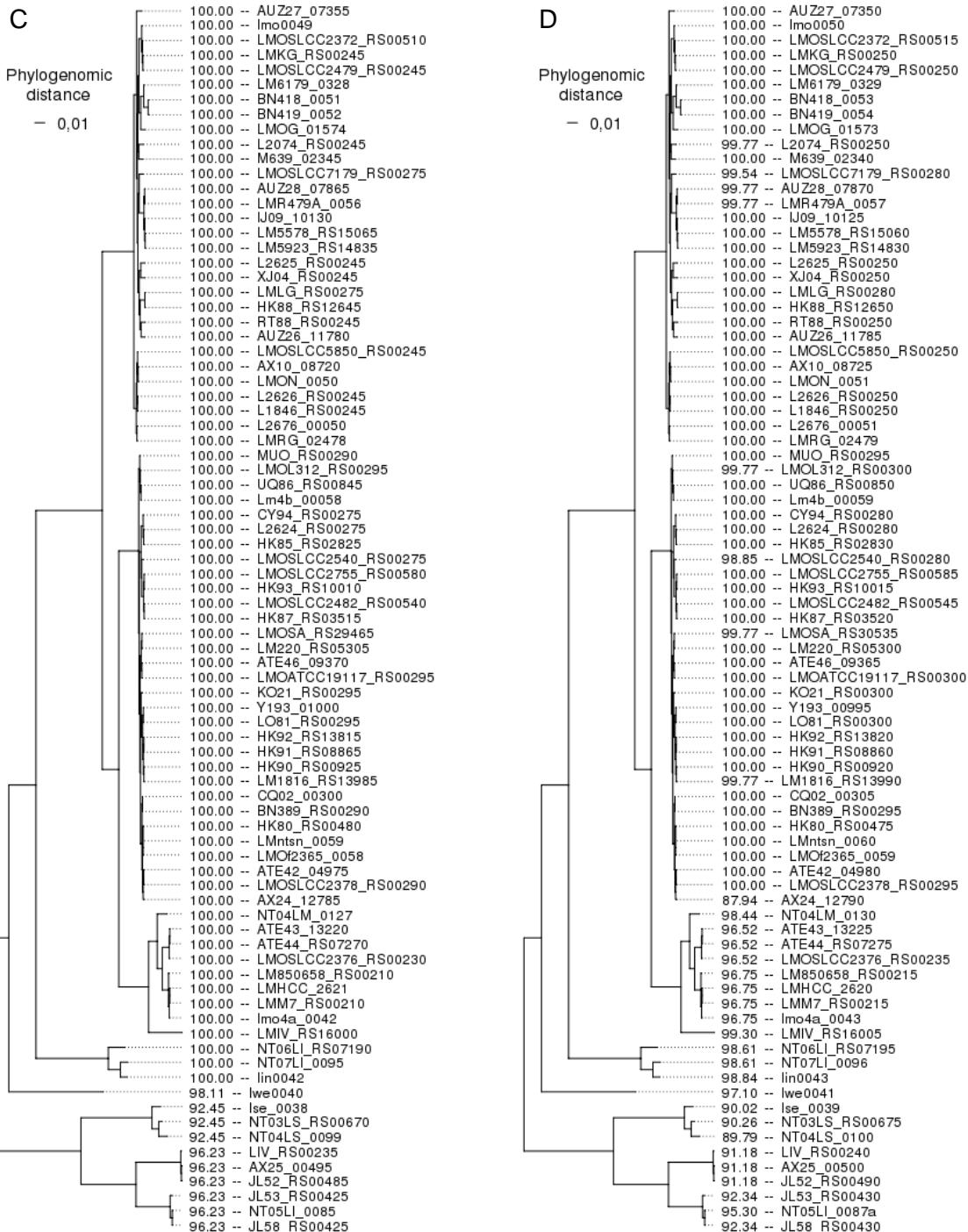
Supplementary Figure S1. DNA-binding region of MouR and FadR. Top: Sequence alignment of winged HTH domain from MouR (*Lm*) and FadR (*E.coli*, PDB ID: 1HW2). Positions of residues responsible for DNA binding in both MouR and FadR are indicated by blue and green dots, respectively. Secondary structure elements observed in the structure of MouR are indicated above the sequence (α -helices with squiggles, β -strands with arrows and turns with TT letters). Strongly-conserved residues are in white on a red background. Partially conserved residues are boxed. Figure was generated in ESPript server (1). Bottom: Superposition of the N-terminal domain of MouR (blue) and FadR (green) bound to DNA. Residues making base-specific contacts in FadR and the structurally overlapping residues in MouR are shown as sticks and labelled.

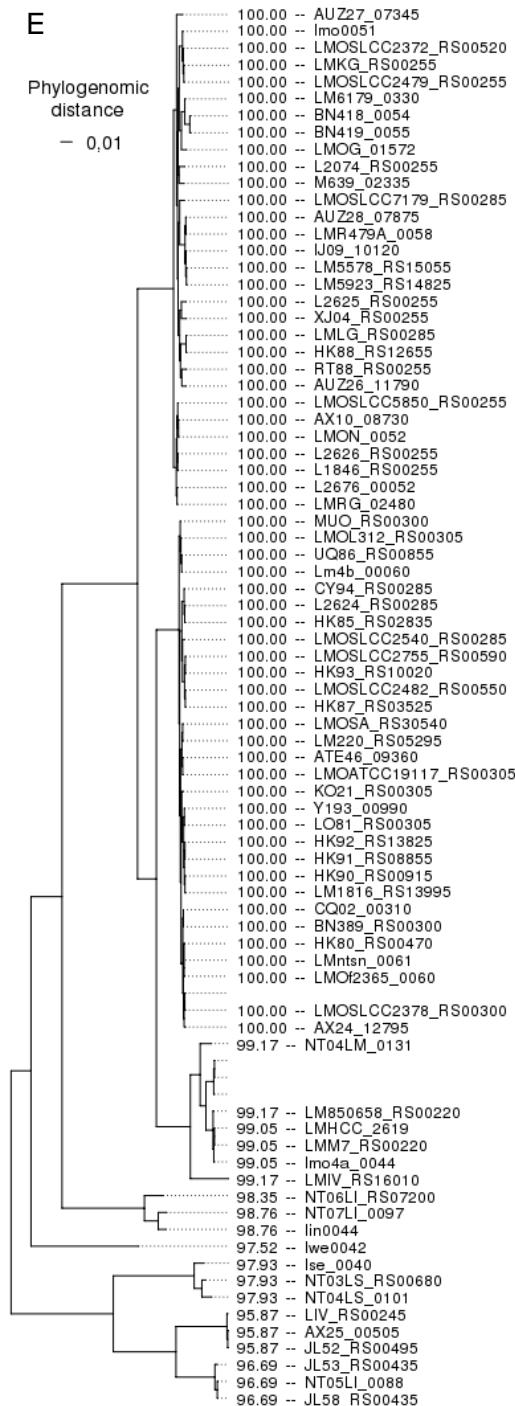
1. Robert,X. and Gouet,P. (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.*, **42**, W320–W324.



Supplementary Figure S2. Expression of WT *mouR* and mutant variants by RT-PCR. Top lane: *mouR* primers; Bottom lane: 16S primers. RT-PCR template scheme: **1-3:** gDNA, cDNA and RNA from *Lm* WT; **4-5:** cDNA and RNA from *Lm* Δ *mouR*+*mouR*(R44/48A); **6-7:** cDNA and RNA from *Lm* Δ *mouR*; **8-9:** cDNA and RNA from *Lm* Δ *mouR*+*mouR*; **10-11:** cDNA and RNA from *Lm* Δ *mouR*+*mouR*(Y80F); **12-13:** cDNA and RNA from *Lm* Δ *mouR*+*mouR*(H133F); **14-15:** cDNA and RNA from *Lm* Δ *mouR*+*mouR*(H134F).







Supplementary Figure S3. *Listeria* ortholog phylogenetic trees of (A) *mouR*, (B) *agrB*, (C) *agrD*, (D) *agrC* and (E) *agrA* genes. Phylogenograms were obtained with Listeriomics (2).

2. Bécavin, C., Koutero, M., Tchitchek, N., Cerutti, F., Lechat, P., Maillet, N., Hoede, C., Chiapello, H., Gaspin, C. and Cossart, P. (2017) Listeriomics: an interactive web platform for systems biology of *Listeria*. *mSystems*, **2(2)**, e00186-16.

Supplementar Table S1 - Plasmids and bacterial strains

Plasmid/Strain	Description	Source
<u>Plasmids</u>		
pMAD	Gram-negative/Gram-positive shuttle vector; thermosensitive replication; Amp ^r Ery ^r	(34)
pMAD(Δ mouR)	pMAD with 5'- and 3'-flanking regions of mouR locus; Amp ^r Ery ^r	This study
pIMK	L. monocytogenes phage-derived site-specific integration vector; Kan ^r	(37)
pIMK(mouR)	pIMK with mouR locus and 5'- and 3'-flanking regions; Kan ^r	This study
pIMK(mouR(R44/48A))	pIMK with mouR locus mutated at R44A + R48A and 5'- and 3'-flanking regions; Kan ^r	This study
pIMK(mouR(Y80F))	pIMK with mouR locus mutated at Y80F and 5'- and 3'-flanking regions; Kan ^r	This study
pIMK(mouR(H133F))	pIMK with mouR locus mutated at H133F and 5'- and 3'-flanking regions; Kan ^r	This study
pIMK(mouR(H134F))	pIMK with mouR locus mutated at H134F and 5'- and 3'-flanking regions; Kan ^r	This study
pET28	Vector system for cloning and expression of recombinant proteins in <i>E. coli</i>	Novagen
pET28a(mouR-6His)	pET28a with mouR locus fused with a 6-Histidin tag at C-Terminal; Kan ^r	This study
pET28a(mouR(R44/48A))	pET28a with mouR locus mutated at R44A + R48A and fused with a 6-Histidin tag at C-Terminal; Kan ^r	This study
<u><i>E. coli</i></u>		
DH5 α	Cloning host strain; F ⁻ Φ 80/ <i>lacZΔM15</i> Δ(<i>lacZYA-argF</i>) U169 <i>recA1 endA1 hsdR17(r_k⁻, m_k⁺) phoA supE44 thi-1 gyrA96 relA1 λ⁻</i>	Life Technologies
BL21(DE3)	F ⁻ <i>ompT hsdS_B</i> (r _B ⁻ m _B ⁻) <i>gal dcm</i> (DE3)	Invitrogen
B834(DE3)	F ⁻ <i>ompT hsdS_B</i> (r _B ⁻ m _B ⁻) <i>gal dcm met</i> (DE3)	Invitrogen
<u><i>Listeria monocytogenes</i></u>		
EGD-e	Wild type; serotype 1/2a	(69)
EGD-e Δ mouR	EGD-e mouR deletion mutant	This study
EGD-e Δ mouR + mouR	EGD-e mouR deletion mutant complemented with pIMK(mouR); Kan ^r	This study
EGD-e Δ mouR + mouR(R44/48A)	EGD-e mouR deletion mutant complemented with pIMK(mouR(R44/48A)); Kan ^r	This study
EGD-e Δ mouR + mouR(Y80F)	EGD-e mouR deletion mutant complemented with pIMK(mouR(Y80F)); Kan ^r	This study
EGD-e Δ mouR + mouR(H133F)	EGD-e mouR deletion mutant complemented with pIMK(mouR(H133F)); Kan ^r	This study
EGD-e Δ mouR + mouR(H134F)	EGD-e mouR deletion mutant complemented with pIMK(mouR(H134F)); Kan ^r	This study
EGD-e Δ agrC	EGD-e agrC deletion mutant	(66)

Supplementary Table S2 - Primers

Name	Sequence (5' - 3')
<i>EMSA</i>	
EMSA-agr-F	AGACCTTAGATCATGTAAACC
EMSA-agr-R	TTTCGAAATCAACACATCCG
EMSA-mouR-F	ATCTATCGGATTAAATGCAGG
EMSA-mouR-R	CATTGGCACTAACTCACC
<i>Plasmid/mutant construction*</i>	
<i>mouR</i> -A	ATT <u>GTCGACCGGCCA</u> ACTTCATTGCG
<i>mouR</i> -B	AT <u>CCATGGCACAAAGTCTGCCCCCT</u> TATGC
<i>mouR</i> -C	CG <u>CCATGGTAAAAAAGCTGTCA</u> CTTTGTGG
<i>mouR</i> -D	CT <u>GAGATCTATTCCCTTGCTGTAGCG</u>
<i>mouR</i> -F	GATGCGGATCGTTATGTGC
<i>mouR</i> -R	GACGATA <u>CGCACTTGATAGGG</u>
pIMK- <i>mouR</i> -F	ACGT <u>CTGCAGTGT</u> CGAAAAGGGATTGC
pIMK- <i>mouR</i> -R	GGC <u>AGTCGACGATTGGGATACAGATTGTGG</u>
<i>mouR</i> (pET28)-F	ACG <u>CCATGGCAAACAAATTAAA</u> CATTAGAT
<i>mouR</i> (pET28)-R	TCCG <u>CTCGAGTCTGTATGCTTGAATCGTT</u>
<i>mouR</i> -R44/48A-F	GAGAATT <u>CGGTGTTAGCGCCTCACCA</u> CTCGAAAAGCCATTGCAACGC
<i>mouR</i> -R44/48A-R	GCGTTGCA <u>ATGGCAAAGCGAGTGGT</u> GAGGCGCTAACACCGAATTCTC
<i>mouR</i> -Y80F-F	GATTGTA <u>TTGATGCGGATCGTTGTGCAATTGATGGAAAC</u>
<i>mouR</i> -Y80F-R	GTTTCCAT <u>CAATTGCACAAACGATCCG</u> CATCAACAATACAATC
<i>mouR</i> -H133F-F	GATT <u>TAGAAAATTATTCGATGCGTTCATG</u> CTTTATTCTTGCTTAAT
<i>mouR</i> -H133F-R	ATTAAG <u>CAAAGAATAAAACGATGAAACG</u> CATCGAAATAATTCTAAATC
<i>mouR</i> -H134F-F	TTAGAAA <u>ATTATTCGATGCGCATTTCG</u> TTTATTCTTGCTTAATTAG
<i>mouR</i> -H134F-R	CTAATT <u>AAGCAAAGAATAAAACGAAATGCGC</u> ATCGAAATAATTCTAA
<i>lmo0443</i> (pET28)-F	ACA <u>ACCAGTGGCAAGACATG</u> CACAAAAAAG
<i>lmo0443</i> (pET28)-R	A <u>ACCCTCGAGCTTAGAAAATCTGTAATGCTT</u>
PL95	ACATAAT <u>CAGTCCAAGTAGATGC</u>
PL102	TAT <u>CAGACCTAACCCAAACCTTCC</u>
<i>RT-qPCR</i>	
qPCR-16S-F	GCGTAGAT <u>ATGTGGAGGAAC</u>
qPCR-16S-R	CAGGCGGAG <u>TGCTTAATG</u>
qPCR- <i>actA</i> -F	CGAGCCTACC <u>AGTAATCC</u>
qPCR- <i>actA</i> -R	CTGATT <u>CGCTTCCCTCTACC</u>
qPCR- <i>agrA</i> -F	GCCTACAC <u>CATCAAGGTATGG</u>
qPCR- <i>agrA</i> -R	ACTTCCGA <u>ATTCCTGAGC</u>
qPCR- <i>agrB</i> -F	TCAGAAAG <u>GAATGGCGGATG</u>
qPCR- <i>agrB</i> -R	CCTGTT <u>ACTAAGGCGATACC</u>
qPCR- <i>agrC</i> -F	AGAAGG <u>TCGTGGATTAGG</u>
qPCR- <i>agrC</i> -R	TCTCT <u>ATCGGTCACTTCG</u>
qPCR- <i>agrD</i> -F	AGAAG <u>AAACATCCATGAAAGTTGC</u>
qPCR- <i>agrD</i> -R	TTCTTG <u>CATTTCACAAATGGACT</u>
qPCR- <i>fruA</i> -F	TTGTA <u>ATTGGTGGAGGTATCG</u>
qPCR- <i>fruA</i> -R	GCGAG <u>TGATCTTGAGGAA</u>

qPCR- <i>inIB</i> -F	AGCACAAACCAAGAAGGA
qPCR- <i>inIB</i> -R	GGCACGGTGATAGTCTCC
qPCR- <i>hly</i> -F	TCGTCCATCTATTGCCAGG
qPCR- <i>hly</i> -R	TTACCGTTCTCCACCATTCC
qPCR- <i>IhrA</i> -F	CGTGTAGTTCATGTCTATGC
qPCR- <i>IhrA</i> -R	ATAAACATTTCCAGCGTTGC
qPCR- <i>lmo0126</i> -F	GTTAGTGTGAATGGTAAGG
qPCR- <i>lmo0126</i> -R	ATCCGTAGTGTATGTTGC
qPCR- <i>lmo0278</i> -F	TGATGAGCCAATCGTTATCG
qPCR- <i>lmo0278</i> -R	CATAAATTCAAGCACCAGTAAGC
qPCR- <i>lmo0463</i> -F	GAGGTGTTCATTCAGAGG
qPCR- <i>lmo0463</i> -R	TAAGGACGAGCACTAACG
qPCR- <i>lmo0641</i> -F	CAATTCAAGCCGAACCTAGG
qPCR- <i>lmo0641</i> -R	CACACTGCCATAAGTAACC
qPCR- <i>lmo0820</i> -F	GGCATCAGATACGAAGAACCAAT
qPCR- <i>lmo0820</i> -R	TTCCAACACCATTGACCAACGATA
qPCR- <i>lmo2251</i> -F	GAGAAAGTGGTCGTAGTTATCG
qPCR- <i>lmo2251</i> -R	GGTTTGTTCGCCATTAGG
qPCR- <i>lmo2650</i> -F	CTCGCTCAATGAATGTGGAT
qPCR- <i>lmo2650</i> -R	TTGTTGACGATGACTACTTGG
qPCR- <i>prfA</i> -F	CGAGTATTAGCGAGAACG
qPCR- <i>prfA</i> -R	GATAACGTATGCGGTAGC

* Restriction sites are underlined

Supplementar Table S3 - Data collection and refinement statistics of MouR.
The numbers in parentheses are for the highest resolution shell

	SeMet (SAD dataset)	Native
Data Collection		
Space Group	P4 ₁	P4 ₁
Wavelength (Å)	0.97915	0.980065
<i>Cell dimensions</i>		
a,b,c (Å)	121.415, 121.415, 60.614	122.445, 122.445, 61.13
α,β,γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	42.89 - 2.793 (2.893 - 2.793)	43.29 - 2.2 (2.279 - 2.2)
Number of observations measured	73122 (7408)	247605 (25155)
Number of unique reflections measured	22034 (2175)	46113 (4603)
Multiplicity	3.3 (3.4)	5.4 (5.5)
Completeness (%)	99.06 (96.80)	99.30 (96.92)
I/σI	10.05 (1.43)	8.62 (0.58)
R _{merge}	0.1029 (0.8352)	0.1159 (1.878)
CC (1/2) (%)	99.6 (59.5)	99.8 (44.4)
Refinement		
R _{work} /R _{free} (%)	22 / 24.84	
Total atoms	3732	
Average B (Å ²)	72.05	
<i>RMSD from standard stereochemistry</i>		
Bond lengths (Å)	0.003	
Bond angles (°)	0.56	
<i>Ramachadran plot statistics</i>		
Favored (%)	97.9	
Allowed (%)	2.1	
Disallowed (%)	0	
PDB Code	6EP3	

Supplementary Table S4 - Accessibility of residues involved in dimerization of MouR. Type of interaction: Hph, hydrophobic; Hyd, hydrogen bond; Sal, salt bridge. Accessibility: ASA, accessible surface area (\AA^2) in the monomer; BSA, buried surface area (\AA^2), i.e., surface engaged in the dimerization interface.

Residue	Location	Interact with	Type	Distance (\AA)	ASA	BSA
Q82	$\alpha 4$	E145 - N146	Hyd	2.96 - 3.25	112.72	72.55
E85	$\alpha 4$	K97	Sal	3.13	95.9	42.66
T86	$\alpha 4$	N146 - Q149	Hyd	3.87 - 3.08	44.53	41.42
I89	$\alpha 4$	I89 - A93	Hph	4.0 - 3.9	112.2	77.83
F90	$\alpha 4$	F90	Hph	3.5	35.63	35.51
A93	$\alpha 4$	I89	Hph	3.6	54.35	30.7
K97	$\alpha 4$	E85	Sal	3.19	70.15	29.75
E145	Loop between $\alpha 6-\alpha 7$	Q82	Hyd	3.14	106.94	15.46
N146		Q82 - T86	Hyd	3.39 - 3.72	40.89	38.97
Y148	$\alpha 7$	Q159	Hyd	3.76	152.67	132.97
Q149	$\alpha 7$	T86	Hyd	3.07	38.57	34.51
I152	$\alpha 7$	I152	Hph	3.7	81.83	55.9